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The Intrinsic Origin of Bioluminescence in the Ascidian, *Clavelina miniata*[1]

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Abstract. Bioluminescence was observed in the ascidian, *Clavelina miniata* (Tunicata: Ascidiacea). This is the first report of luminescence in ascidians. A green light was evoked by mechanical stimulation, by increasing the concentration of K^+ ions, or by hypotonic conditions. The source of the light was a type of tunic cell (cell type II). This luminescence is attributed to an intrinsic system, not to bacterial symbionts.

Introduction

Bioluminescence in tunicates has been reported for pelagic species of the Thaliacea and Larvacea (Harvey, 1952; Herring, 1978), and there are two old reports of luminescence in ascidians: Landsborough (1842) in *Botryllus schlosseri* and Will (1844) in *Ciona (Phallusia) intestinalis*. The luminescence of these two ascidians is believed to have been caused by infections of luminous bacteria (Skowron, 1926; Harvey, 1952). However, recently we observed bioluminescence in *Clavelina miniata*, a colonial member of the Ascidiacea inhabiting sublittoral rocky shores (Watanabe and Tokioka, 1973). Our studies indicate that the bioluminescence of this ascidian is produced intrinsically, not by symbiotic bacteria.

Materials and Methods

Clavelina miniata colonies were reared on glass plates in rafts immersed in an inlet near the Shimoda Marine Research Center during 1983–1985. Fresh colonies were transferred from the rafts to aquaria in the laboratory for each experiment. Although the ambient seawater temperature range was 12°C–25°C, experiments were conducted at 18°C–20°C. Many individuals of this species grow in a dense aggregation (social type), but they never form a colony in the strict sense because newly formed individuals become physically separated by strobilization of the abdomen (Kamijo and Watanabe, unpub.) before the branchial aperture opens. Thus, individuals can be easily removed from a glass plate. Each individual is elongate, about 10 mm long in the extended state, and consists of a thorax and an abdomen enclosed in a gelatinous, transparent tunic (Fig. 1), which can be removed easily.

Preliminary observations showed that a remarkable green light was emitted from the whole tunic when placed in a hypertonic solution such as 2 *M* KCl or 2 *M* NaCl, or in a hypotonic solution. Also, mechanical stimulation of any point on the tunic resulted in localized light emission. Mechanical stimulation consisted of touching the tunic with a fine needle. The effects of these stimuli were then examined more thoroughly.

To determine the effect of K^+ ions or Na^+ ions while excluding the effect of osmotic pressure, mixed solutions of 0.54 *M* KCl and 0.54 *M* NaCl, isotonic with seawater, were prepared. The ratio of KCl to NaCl was varied from 0.0 to 1.0, at intervals of 0.1. For each ratio of solution, an individual was immersed in 1.0 ml of the mixed solution. Four individuals were examined. For each individual, light emission was recorded with an LKB Wallac luminometer, at 1-min intervals over a 10-min period, and the maximum value of relative light intensity was plotted.

Long duration light emission from the whole tunic was also observed when individuals were placed in a hypo-

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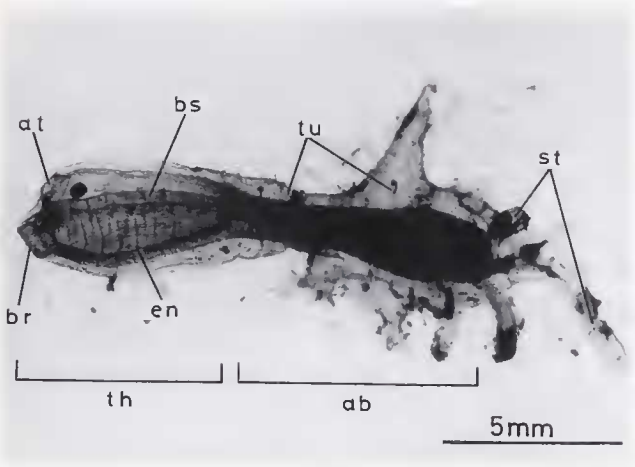

Figure 1. *Clavelina miniata*, growing on a glass plate, consists of a thorax and an abdomen enclosed in a transparent tunic. ab: abdomen; at: atrial siphon; br: branchial siphon; bs: branchial sac; en: endostyle; st: stolon; th: thorax; tu: tunic.

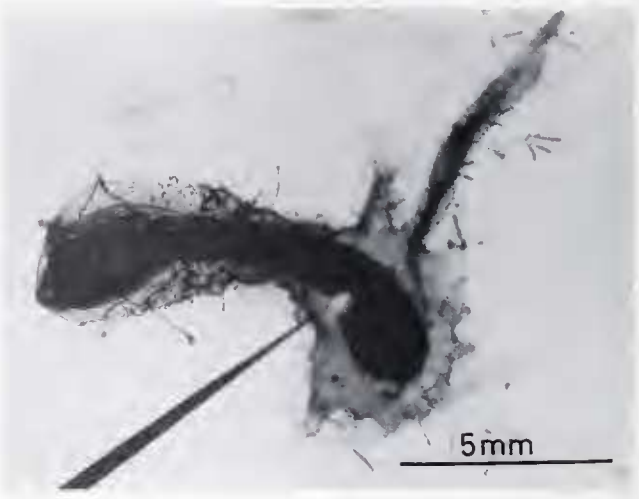

Figure 2. Luminescence of *Clavelina miniata* evoked by mechanical stimulation. The individual is touched by a fine needle. Note the strong luminescence emitted locally on tunic.

tonic solution. Three individuals were examined. Each was immersed in distilled water and records of relative intensity of light emission were made at 1-min intervals, until light emission was no longer detectable.

The thoracic tunic was chosen for microscopic observations, because of its high transparency. The tunic on a glass slide kept at room temperature begins to glow as the seawater evaporates, thus increasing the osmolarity. To establish the source of light emission, the position of flashes in the tunic was observed through an unilluminated microscope with a video camera and photomultiplier (Hamamatsu Photonics C-1000) and recorded on video film (SONY U-Matic VO-5800 Video Recorder). When light was no longer emitted, the tunic cells were illuminated and videotaped with the same apparatus. Cells in the tunic were examined using a Nikon microscope with Nomarski differential interference contrast optics.

Photographs were taken using Kodak Tri-X (ASA 400) or Kodak Plus-X (ASA 50) film.

Results

Stimulation evoking luminescence

(a) Mechanical stimulation. Mechanical stimulation with a fine needle could evoke luminescence from any part of the tunic of an intact individual. A strong green luminescence appeared locally at the site of stimulation (Fig. 2). The duration varied from 2 to 5 s.

(b) Change of ratio of K^+ ions and Na^+ ions. Relative light intensity of light emission was lowest for a K^+/Na^+ ratio of 0.1, and became higher as the proportion of K^+ ions was increased, especially >0.7 (Fig. 3).

(c) Hypotonic condition. In distilled water, the whole tunic of an individual began to emit a strong green light (Fig. 4). The intensity of light emitted varied for each individual, but reached a maximum after 2–5 min. The duration of light emission varied from 15 to 30 min (Fig. 5).

Source of light emission

Under a stereomicroscope (20×), the light emitted from the tunic following the stimuli mentioned above

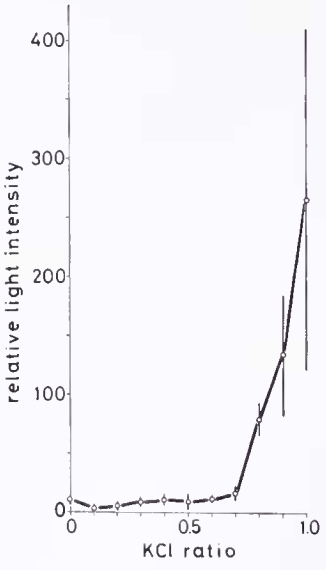

Figure 3. Mean maximum value of relative light intensity of luminescence emitted from the individuals during 10 minutes in solutions with different ratios of 0.54 *M* KCl and 0.54 *M* NaCl. The ratio of KCl to NaCl was varied from 0.0 to 1.0 at 0.1 intervals. Four individuals were examined for each ratio. Vertical bars indicate standard errors of the mean.

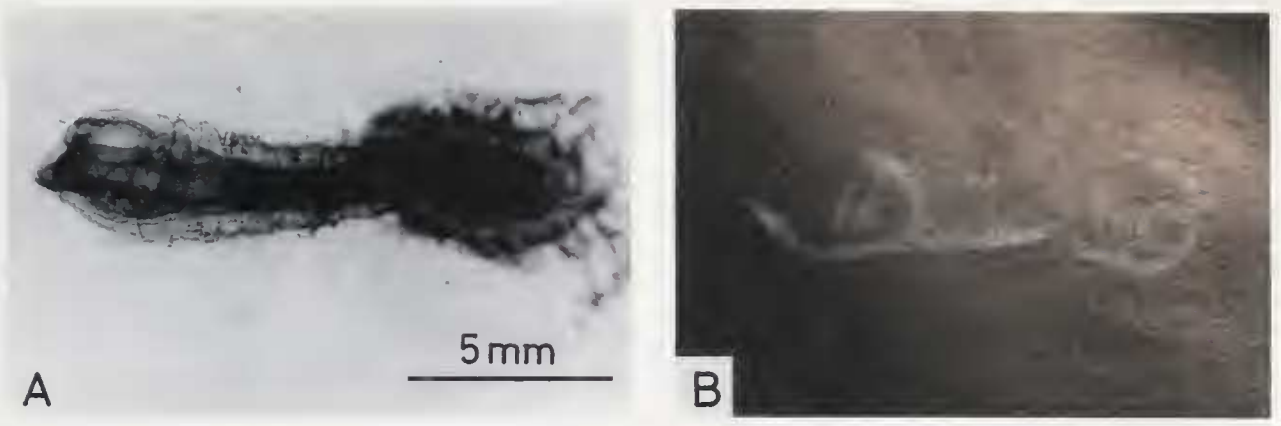

Figure 4. Luminescence of *Clavelina miniata* evoked by hypotonic conditions. (A) An intact individual in seawater; (B) an individual in distilled water, emitting light from the tunic continuously over a long duration.

was discerned to be groups of point-source lights in the tunic. The long duration light emission is due to the successive flashes of point-source lights over a period of time rather than the continuous luminescence of all the sources. On the other hand, the light emission by mechanical stimulation is a result of local luminescence of the point-sources at the stimulated site of the tunic. The point-source lights are apparently from the tunic cells. Cells are distributed densely in the tunic (Fig. 6A). Under examinations with Nomarski optics, these cells were classified by their morphological characteristics into three types:

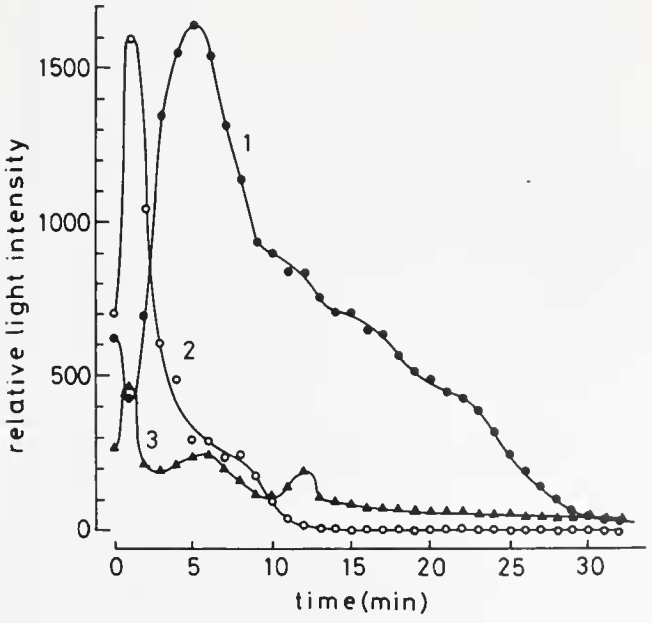

Figure 5. Changes in the relative light intensity of luminescence emitted by three individuals under hypotonic conditions. Numbers in the figure indicate different individuals.

I. Cells including 10–15 large amorphous vacuoles. Cell size: 20–25 μm long and 10–15 μm wide (Fig. 6B).

II. Flat cells including 1–10 spherical granules of 0.7–1.0 μm diameter. Cell size: 15–20 μm long and 7–10 μm wide (Fig. 6C).

III. Cells filled with many oval granules of 0.7–1.8 μm in the long axis. Cell size: 15–20 μm long and 10–15 μm wide (Fig. 6D).

Through an unilluminated light microscope, light emission appeared from a point-source, scattering strongly from the center before fading within 0.5–1.0 s (Fig. 7B–D). Comparing these figures with a figure of tunic cells (Fig. 7A), it is clear that the light was emitted from a definite type of cell: cell type II. In numerous preparations, we observed no light emission from other cell types.

Discussion

Although this is the first report of intrinsic bioluminescence in ascidians, there are several papers describing the luminescence of pelagic species in the Thaliacea and Larvacea.

Generally, three types of animal luminescent responses can be distinguished. These are extracellular luminescence, intracellular luminescence, and luminescence due to symbiotic bacteria (Nicol, 1960). In the case of luminescence due to bacterial symbionts, the presence of luminous bacteria can be established by microscopic observation, culturing, continuous light emission, and the occurrence of bacterial luciferase (Nicol, 1960; Leisman *et al.*, 1980; Nealson and Hastings, 1980). However, in *Pyrosoma* (Tunicata: Thaliacea), the distinction between symbiotic and intrinsic light emission was not easily made. It has been suggested that the source of intermittent luminescence is not symbiotic bacteria (Harvey,

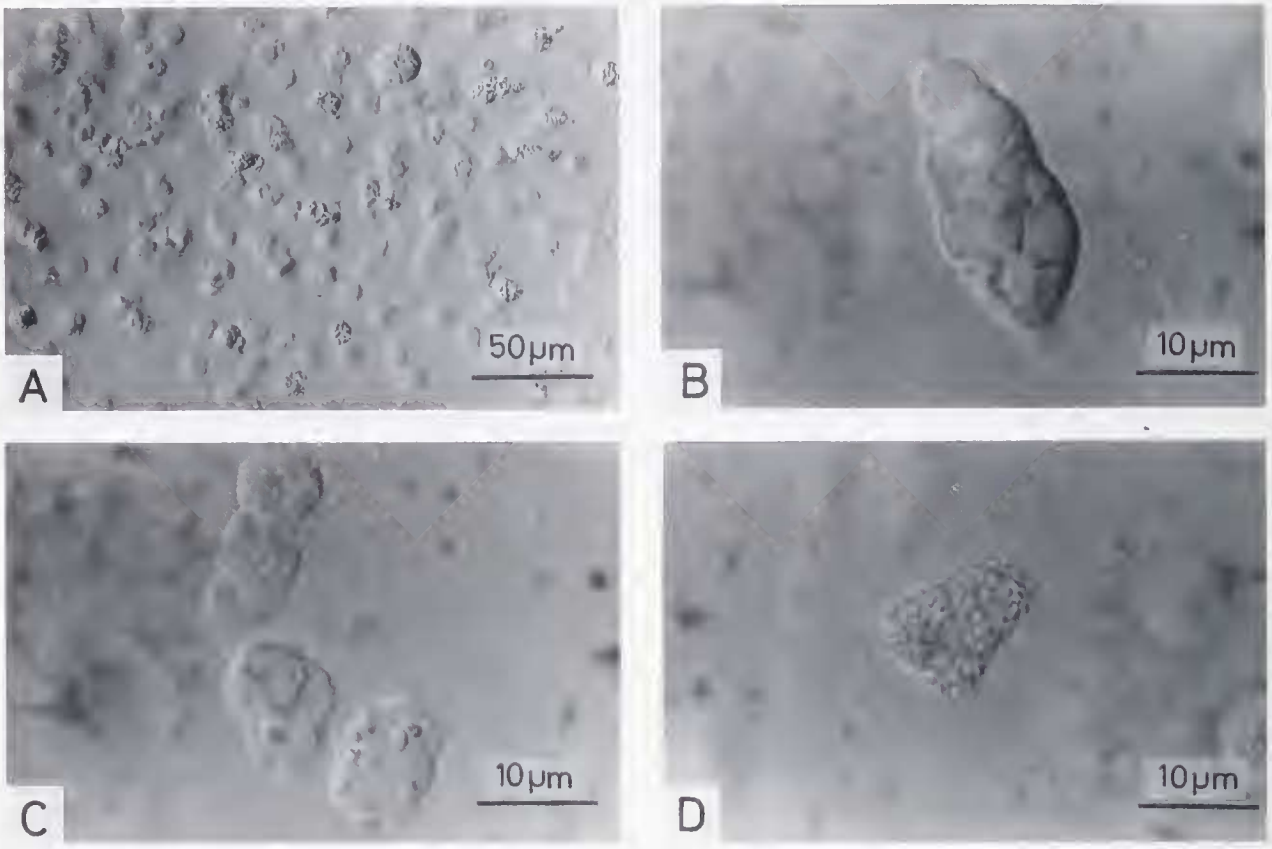

Figure 6. Portion of the thoracic tunic of *Clavelina miniata* viewed with Nomarski optics showing the distribution of cells in the tunic (A); a tunic cell of type I (B); tunic cells of type II (C); and a tunic cell of type III (D).

1952). According to Nicol (1960), the luminescence of *Pyrosoma* apparently differed from that of luminous bacteria because it was intermittent, excitable by tactile, electrical, and photic stimuli, and quenchable by illumination. However, bacteria-like bodies packed in the luminous organ have been observed (Mackie and Bone, 1978), and bacterial luciferase has been found in it (Leisman *et al.*, 1980; Nealson and Hastings, 1980). The luminescence of *Pyrosoma* may be controlled by the packaging of bacterial symbionts within the host cells (Mackie and Bone, 1978; Anctil, 1979; Nealson and Hastings, 1979, 1980).

On the other hand, in the larvaceans, oikopleurids possessing oral glands produce endogenous light upon mechanical stimulation (Fredriksson and Olsson, 1981; Galt *et al.*, 1985). In *Oikopleura dioica* and *O. labradoriensis*, light was produced from 1–2 μm clusters of fluorescent granules (Sykes, 1980; Galt and Sykes, 1983). However, the light emitting mechanism in larvaceans is unknown (Galt and Sykes, 1983) and bacterial luciferase has not been found (Galt, 1978; Hastings, 1983). The relationship between the luminescent mechanisms of *Clavelina* and larvaceans is unknown, although they share the characteristics that light can be evoked from a point-source by mechanical stimulation.

The mechanism of luminescence in *Clavelina* is probably caused by an intrinsic mechanism. The apparently continuous luminescence was found to be the result of successive flashes of point sources of light. Furthermore, the emission of light could be caused by various modes of stimulation such as mechanical stimulation, increase of extracellular K^+ ions, and hypotonic conditions.

The results obtained in the observation through the unilluminated microscope show that one type of tunic cell of *Clavelina*, the type II cell, luminesces when stimulated appropriately. This type of cell can be easily discriminated from the other two by its morphological characteristics. We do not yet know the chemical nature of the luminescence.

In the ascidian *Clavelina*, light emission was strong when the K^+ ratio was high, so the luminescence may be caused by the asymmetrical distribution of anions and cations across the cell membrane. This type of luminescence has been reported for the coelenterates, *e.g.*, *Vere-*

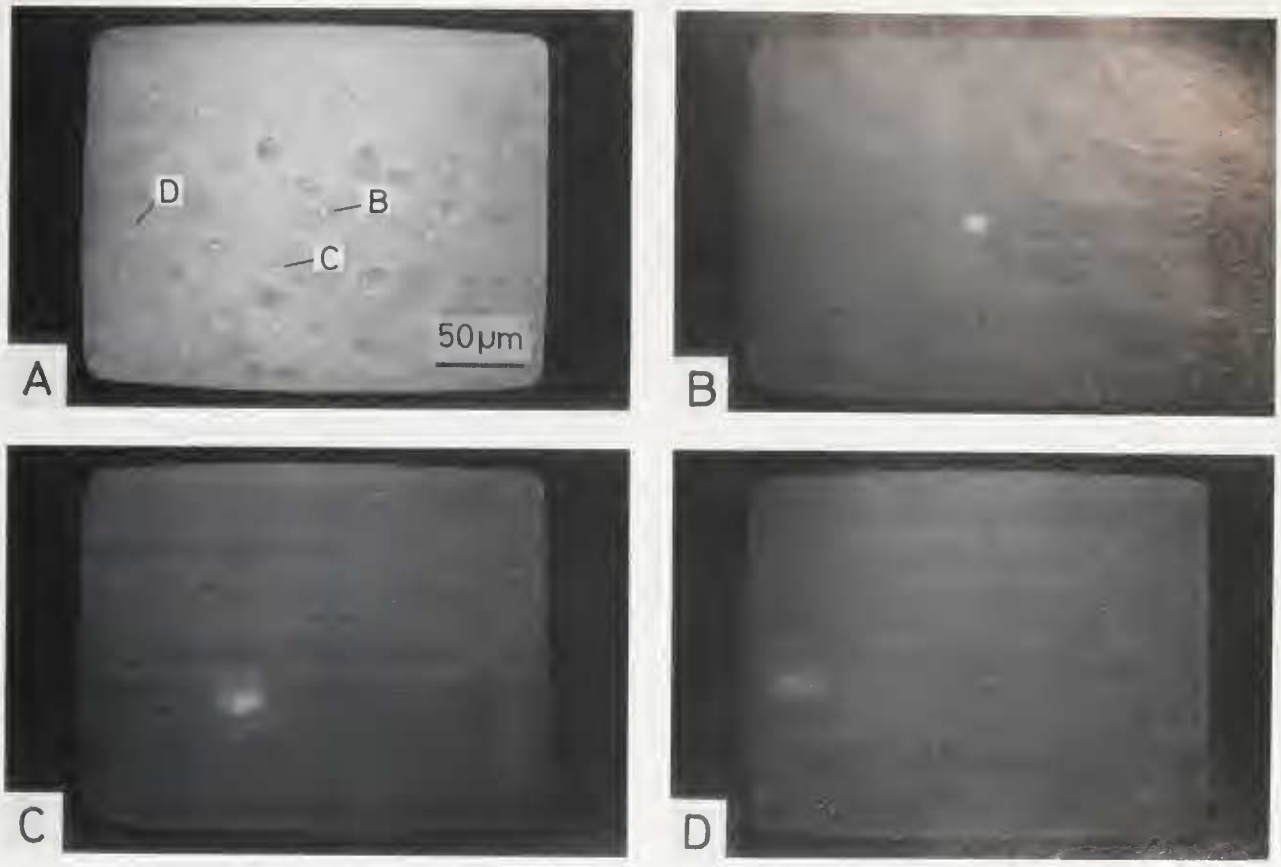

Figure 7. The source of light emission in the tunic. Tunic cells recorded on video tape through an illuminated microscope showing cell types I, II, and III (A). Individual type II cells (B, C, D as indicated in A) emitting light recorded on video tape through a fixed unilluminated microscope with a video camera and a photomultiplier (B–D).

tillum and *Renilla* (Henry and Michelson, 1978; Henry and Ninio, 1978). In this case, an asymmetrical distribution of cations and anions on the membrane of the lumisomes was attributed to an influx of Ca^{2+} ions, which triggers luminescence. Ca^{2+} ions can pass through the cell membrane if there is a higher proportion of Na^{+} ions inside the cell, and a higher proportion of K^{+} ions outside. Mechanical stimulation may also change the ion permeability of the membrane, inducing luminescence.

The luminescence observed in hypotonic conditions may be caused by the rupture of cell membranes after osmotic uptake of water, thereby releasing the luminous substance. A similar phenomenon is known in the lumisome system: lumisomes in distilled water can emit light (Anderson and Cormier, 1973).

Further experiments are required to clarify the chemical nature of the luminous substance and the mechanisms of the luminescence, as well as the fine structure of the type II cell.

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